The analysis of illicit 25X-NBOMe seizures in Western Australia

1 | INTRODUCTION

In recent years there have been numerous publications relating to the identification of new and novel illicit drugs from a wide range of chemical classes; including cathinones, tryptamines and phenylethylamines, along with the identification of novel synthetic cannabinoids.1-4 One group of compounds that has received widespread attention is the N-(2-methoxy) benzyl derivatives of well-known phenylethylamines such as 2C-I and 2C-C.5-8 This class of compounds is generally named using the abbreviation for the phenylethylamine (such as 2C-I) combined with the NBOMe acronym representing the N-(2-methoxy)benzyl moiety (for example 2C-I-NBOMe, also known as 25I-NBOMe).7,9

Several recent deaths attributed to NBOMe-related compounds have received coverage in the press both in Australia and internationally. There have also been numerous publications reporting fatal intoxications associated with NBOMe compounds highlighting the dangerous nature of these compounds.10-15 Since mid-2014, 25I-NBOMe, 25C-NBOMe, and 25B-NBOMe have been specifically listed under the Misuse of Drugs Act 1981 in Western Australia as controlled substances.16 These three compounds have been listed by the United Nations Office on Drug and Crime (UNODC) under Schedule I of the Convention on Psychotropic Substances of 1971 since March 2015.17 To date, the reporting of the quantitation of NBOMe compounds identified on blotter paper by forensic science laboratories has been almost non-existent, with just one publication reporting the quantitation of just three individual pieces of blotter paper.18 The majority of the literature regarding the quantitation of NBOMe compounds report the levels found in blood and urine samples. Anecdotal evidence from users found on internet forums, such as Bluelight and drugs-forum.com, generally state that an average dose is approximately 500 μg.19,20

Seizures submitted for analysis to this laboratory found to contain NBOMe compounds were in the majority of cases in the form of blotter paper, with only a few cases in which an NBOMe compound was identified in tablets. Examples of seizures submitted for analysis are shown in Figure 1.

This paper summarizes the results of over 100 seizures containing NBOMe compounds submitted to ChemCentre for analysis by the Western Australia Police. All the samples submitted were seized within Western Australia between January 2014 and February 2016.

2 | EXPERIMENTAL

2.1 | Standards and reagents

Methanol (Optima™ LC-MS grade, purity ≥99.9%) was supplied by Fisher Chemical (Scoresby, Victoria, Australia). Pyrrolidine (purity ≥99.5%) was supplied by Sigma-Aldrich (St Louis, MO, USA). Glacial acetic acid was supplied by Ajax Finechem (Taren Point, Australia). MilliQ grade water was prepared in house using a MilliPore MilliPore Ltd fitted with a LiquiPure 1 cartridge supplied by EVOQUA (Bayswater, Victoria, Australia). The 0.2 μm hydrophilic PTFE filters were supplied by Merck Millipore Ltd (Tullagreen, Carrigtwohill, Ireland). Certified reference materials for 25B-, 25I-, 25T- and 25C-NBOMe were supplied by Lipomed (Arlesheim, Switzerland). Certified reference materials for 25D- and 25E-NBOMe were supplied by National Measurement Institute (Sydney, Australia). Certified reference materials for 25I-NBMD and 25I-NBF were supplied by Cerilliant (Round Rock, TX, USA). Certified reference materials for 25H- and 25G-NBOMe were supplied by Cayman Chemical (Ann Arbor, MI, USA).

2.2 | Sample preparation

2.2.1 | Paper tabs

To a single piece of blotter paper (paper tab) in a glass disposable test tube, 1 mL of methanol was added and the sample vortexed for 30 seconds followed by sonication for 10 minutes. The sample was diluted with water 6-fold using a Hamilton Microlab Series 500 auto diluter (Grace Davison Discovery Sciences, Reno, NV, USA). The resulting dilution was then filtered through a 0.2 μm hydrophilic PTFE filter by vacuum filtration and analyzed by liquid chromatography coupled to a photodiode array detector using an auto injector with a 1 μL injection volume.

2.2.2 | Tablets

A single tablet was crushed and homogenized using a mortar and pestle and approximately 30 mg of the powdered tablet weighed into a glass disposable test tube and dissolved in 5 mL of MilliQ-grade water. The sample was then vortexed for 30 seconds, filtered through a 0.2 μm hydrophilic PTFE filter by vacuum filtration and then analyzed by liquid chromatography using an auto injector with a 1 μL injection volume.
2.3 | Instrumentation

Chromatographic separation was achieved using a Waters Acquity H-Class Ultra Performance Liquid Chromatography (UPLC) system coupled to a Waters Acquity UPLC photodiode array (PDA) λ detector (Milford, CT, USA). The analytical column was a Waters Acquity UPLC BEH C18 1.7 μm 2.1 mm x 100 mm held at 50°C (Waters, Wexford, Ireland). An isocratic solvent elution system consisting of 70% methanol and 30% water with 0.2% pyrrolidine and 0.1% glacial acetic acid with a flow rate of 0.35 mL/min and an injection volume of 1 μL was used. The PDA scanned wavelengths from 210 to 400 nm with a scanning rate of 20 points/second, with the 214 -nm wavelength used for quantification.

2.4 | Method validation

The linearity of the method was assessed using multiple 6-point calibrations over a range of 20 μg/mL to 330 μg/mL. Correlation coefficients (r²) values were determined by linear regression. The instrument repeatability was assessed by multiple (n=7) injections of each calibration level and calculating their mean and %RSD values. The extraction efficiency for paper tabs was assessed by spiking blank paper tabs and analyzing these tabs using the defined method. The limit of detection was defined as the lowest concentration giving a peak with signal-to-noise ratio >3 and with the UV spectrum showing a λmax consistent with the expected value.

3 | RESULTS

A selection of 25X-NBOMe compounds, for which a certified reference material was available at the time, were analyzed using the defined procedure with their retention times listed in Table 1.

Only 4 of the compounds listed in Table 1 (25H-, 25C-, 25I- and 25B-NBOMe) have been encountered in samples submitted for analysis to laboratory and it is these compounds that form the basis of this study. Baseline separation of these 4 compounds was achieved using the defined procedure as shown in Figure 2.

3.1 | Blotter paper (paper tabs)

A summary of the results obtained from blotter paper (paper tabs) NBOMe seizures is summarized in Table 2. Results indicate that the average amount on each tab, for 25C-NBOMe and 25B-NBOMe to be approximately 700 μg/tab, whilst for 25I-NBOMe this value was slight lower at 600 μg/tab. These values are comparable to what has been reported anecdotally by users of drug forums. There was a significant difference observed between the highest and lowest amounts per tab recorded for each of the three NBOMe compounds.

For 25I-NBOMe and 25B-NBOMe there is an approximately 11-fold difference between the highest and lowest amount detected per tab, with the lowest amount detected being 110 and 120 μg, respectively, whilst the highest amounts per tab were approximately 1200 and 1500 μg, respectively. For 25C-NBOMe, there was only a 2-fold difference between the lowest amount and the highest amount recorded. This potentially equates to a tenfold difference in the dose encountered for a single paper tab on separate occasions for a user. The wide range in concentrations per tab highlights the high risk with taking blotter papers believed to contain NBOMe compounds and the potential for adverse effects.

In most of the blotter papers analyzed more than one NBOMe compound was detected on the blotter paper. Typically, there was one main component (for example 25I-NBOMe), with other NBOMe compounds (eg. 25B-NBOMe and 25C-NBOMe) also detected between 30 and 100 μg per blotter. The presence of 25H-NBOMe as a minor component on blotter paper is likely to have originated as part of the synthesis of the main halogenated NBOMe compound on the blotter paper and is unlikely to have been intentionally added to the blotter paper.

For the paper tabs that were found to contain 25I-NBOMe as the main component, in 50% of these cases it was the sole compound identified, of the remaining cases 25C-NBOMe was identified as a minor component in 45%, whilst 25B-NBOMe was identified as a minor component in just 5%. For paper tabs that contained 25B-NBOMe as the main component, in two-thirds of the samples analyzed it was the sole NBOMe compound identified, with 25I-NBOMe detected as a minor component in the remaining

![FIGURE 1 A selection of the different varieties of paper tabs and tablets submitted for analysis](Colour figure can be viewed at wileyonlinelibrary.com)

| **TABLE 1** A selection of 10, 25X-NBOMe compounds analyzed using this method and their retention time |
|-----------------------------------------------------|-----------------------------------------------------|
| **Retentlon Time (mins.)** | **Retention Time (mins.)** |
| 25H-NBOMe | 1.9 |
| 25C-NBOMe | 2.6 |
| 25D-NBOMe | 2.7 |
| 25T-NBOMe | 2.9 |
| 25I-NBMD | 2.9 |
| 25B-NBOMe | 3.0 |
| 25I-NBF | 3.2 |
| 25G-NBOMe | 3.4 |
| 25I-NBOMe | 3.5 |
| 25E-NBOMe | 3.8 |
one-third of the samples. Interestingly 25C-NBOMe was not detected on any of the paper tabs found to contain 25B-NBOMe as the main component.

Paper tabs found to contain 25C-NBOMe as the main component were less common compared to those paper tabs with 25I- or 25B-NBOMe, and in less than 10% of these cases it was sole NBOMe compound detected. This contrasts with paper tabs containing 25B-NBOMe and 25I-NBOMe, which were identified as the sole NBOMe compound in at least 50% of their respective sample populations. In over 50% of paper tabs found to contain 25C-NBOMe was the main component, 25I-NBOMe was also identified.

The presence of additional NBOMe compounds could indicate that the manufacture of these compounds or the preparation of the paper tabs is occurring at one location for a range of different NBOMe compounds. Given the amount per tabs for the minor components were below 100 μg, it is unlikely that these compounds have intentionally been added to the paper tab.

The concentration range distribution for 25C-NBOMe, 25B-NBOMe and 25I-NBOMe, which were the main components on the paper tabs analyzed, is shown in Figure 3. Of the tabs analyzed, 25H-NBOMe was not identified as the main component in any of the samples. The most commonly encountered compound was 25I-NBOMe, accounting for just over 50% of all the cases analyzed. The figure shows the large range in the amounts detected per tab which highlights the risk of adverse drug effects given the potentially large differential between dosages. An additional risk of experiencing adverse drug effects, is paper tabs containing NBOMe compounds may have been sold and/or purchased under the pretense of being LSD, which generally has a much lower dosage per paper tab, with the average LSD content per tab for samples submitted to ChemCentre for analysis approximately 40 μg.

The values for the quantitation of three paper tabs reported in literature are consistent with those seen in this study. All three of the paper tabs were found to possess one major component along with multiple minor components, which was also seen in this study. The reported 25C-NBOMe and 25I-NBOMe contents were slightly lower than the average values reported in this study. The 25B-NBOMe content reported was higher than the maximum reported

### Table 2

**Statistical Summary of Blotter Paper (paper tabs) Samples (n=119)**

<table>
<thead>
<tr>
<th></th>
<th>25C-NBOMe</th>
<th>25B-NBOMe</th>
<th>25I-NBOMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average (ug/tab)</td>
<td>693</td>
<td>740</td>
<td>642</td>
</tr>
<tr>
<td>Maximum (ug/tab)</td>
<td>1100</td>
<td>1480</td>
<td>1193</td>
</tr>
<tr>
<td>Minimum (ug/tab)</td>
<td>553</td>
<td>123</td>
<td>114</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>176</td>
<td>363</td>
<td>298</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>25.4</td>
<td>49.1</td>
<td>46.4</td>
</tr>
<tr>
<td>Median (ug/tab)</td>
<td>630</td>
<td>850</td>
<td>591</td>
</tr>
</tbody>
</table>
in this study. This further highlights the variability in the NBOMe content on paper tabs.

### 3.2 Tablets and powders

Two NBOMe compounds, 25C-NBOMe and 25I-NBOMe, have been encountered at the laboratory in tablet form to date, with the results for analysis summarized in Table 3. The average content by weight of 25C-NBOMe and 25I-NBOMe in the tablets and powder analyzed was between 0.3% and 0.4%. The highest content by weight for 25C-NBOMe and 25I-NBOMe were 0.6% and 0.7%, respectively. As was the case for paper tabs, there was a wide range between the highest and lowest content of 25C-NBOMe (0.6% and 0.10% by weight, respectively) and 25I-NBOMe (0.7% and 0.05% by weight, respectively). The tablets analyzed were also commonly found to contain caffeine and in some cases trace amounts of 3, 4-methylenedioxymethylamphetamine (MDMA).

The most prevalent tablet type submitted for analysis by total weight and number of exhibits were round blue tablets with a "Batman" motif, as shown in Figure 1. The tablets contained 25I-NBOMe with an average content by weight of 0.49%. The tablets also contained caffeine. The average unit weight for these tablets was 0.24 grams. This equates to approximately 1176 μg 25I-NBOMe per tablet, which is very close to the highest amount detected for 25I-NBOMe on a single paper tab. Other tablets encountered include red unmarked tablets, yellow star shaped tablets and cream-colored tablets bearing a "Louis Vuitton" logo.

### 3.3 Method validation

The UPLC method was shown to have good linearity for the three main NBOMe compounds of interest (25C-, 25I- and 25B-NBOMe). Six-point calibration curves conducted over multiple days across a range of 20 μg/mL to 330 μg/mL were used to assess linearity using regression analysis. The method was shown to be linear across this range with coefficient of determination ($r^2$) values of 0.999 or greater. The method was shown to be linear up to 1800 μg/mL. All the calibrators used to assess for instrument repeatability had %RSD values less than 5%.

A 6-point calibration is performed with every analysis and the reported concentrations for each component in the standard must be within 10% of their expected values for results to be acceptable. The extraction procedure defined above was shown to be quantitative (>88%) for paper tabs. Due to the small quantity of reference material available, other extraction techniques were not assessed. The limit of detection for the three main NBOMe compounds using this procedure was 5 μg/mL.

### 4 Conclusion

This paper describes a rapid method for the quantitation of a range of several commonly encountered NBOMe compounds. The results from over 100 paper tabs analyzed by ChemCentre show the average amount per paper tab to be approximately 700 μg; however, there was significant difference between the lowest and highest amounts detected per paper tab. The average NBOMe content in the tablets analyzed was approximately 0.3% and 0.4% by weight for 25I-NBOMe and 25C-NBOMe respectively. The significant variability in the content of the paper tabs and tablets analyzed highlights the high chance of adverse effects given the potential inconsistencies in dosages.
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